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Structural basis for tumor necrosis factor blockade with the therapeutic antibody golimumab

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Abstract

Tumor necrosis factor α (TNFα) is a proinflammatory cytokine, and elevated levels of TNFα in serum are associated with various autoimmune diseases, including rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn's disease (CD), psoriasis and systemic lupus erythaematosus. TNFα performs its pleiotropic functions by binding to two structurally distinct transmembrane receptors, TNF receptor (TNFR) 1 and TNFR2. Antibody-based therapeutic strategies that block excessive TNFα signaling have been shown to be effective in suppressing such harmful inflammatory conditions. Golimumab (Simponi®) is an FDA-approved fully human monoclonal antibody targeting TNFα that has been widely used for the treatment of RA, AS and CD. However, the structural basis underlying the inhibitory action of golimumab remains unclear. Here, we report the crystal structure of the Fv fragment of golimumab in complex with TNFα at a resolution of 2.73 Å. The resolved structure reveals that golimumab binds to a distinct epitope on TNFα that does not overlap with the binding residues of TNFR2. Golimumab exerts its inhibitory effect by preventing binding of TNFR1 and TNFR2 to TNFα by steric hindrance. Golimumab does not induce conformational changes in TNFα that could affect receptor binding. This mode of action is specific to golimumab among the four anti-TNFα therapeutic antibodies currently approved for clinical use.

Keywords: tumor necrosis factor α (TNFα); autoimmune disease; therapeutic antibody; golimumab; X-ray crystallography, competitive inhibition, steric hindrance

A 50-75-word statement, written for a broader audience, outlining the importance and/or impact of the work presented in the manuscript:

The anti-TNFα therapeutic antibody golimumab (Simponi®) received FDA approval in 2009 and has demonstrated robust clinical efficacy in the treatment of rheumatic disease; however the structural basis underlying its inhibitory action remains unclear. In this study, we report the crystal structure of the TNFα:golimumab complex and reveal the mode of action of golimumab, which is different from that of the other FDA-approved anti-TNFα antibodies, infliximab (Remicade®), adalimumab (Humira®) and certolizumab pegol (Cimzia®).

Introduction

Tumor necrosis factor α (TNFα) is a pleiotropic cytokine involved in regulating diverse bodily functions, including cell growth modulation, inflammation, carcinogenesis, viral replication, septic shock and autoimmunity.[1] TNFα performs its functions by binding to two structurally distinct membrane receptors, TNFR1 (also known as p55) and TNFR2 (p75).[2] TNFα signaling via TNFR1 is predominantly associated with inflammation and apoptosis. In contrast, TNFR2 is mainly implicated in regulation of T-cell migration and tissue regeneration. Because of its pleiotropic actions, the biosynthesis of TNFα is under strict control of multiple and complex regulatory mechanisms, including gene transcription, mRNA turnover, translation and intracellular signaling of the protein. Dysregulation of TNFα production can result in elevated serum TNFα levels and the development of various inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn's disease (CD), psoriasis and systemic lupus erythaematosus.[3]

Recently, therapeutic strategies that block excessive TNFα signaling have emerged to suppress these harmful inflammatory conditions. To date, the following five molecules have been approved by the FDA as effective TNFα inhibitors for clinical use and led to significant advances in autoimmune inflammatory disease therapy: infliximab (Remicade®; humanized chimeric monoclonal antibody (mAb) of the isotype IgG1), adalimumab (Humira®; fully human mAb of the IgG1/κ isotype), certolizumab pegol (Cimzia®; PEGylated humanized Fab fragment), golimumab (Simponi®; fully human mAb of the IgG1/κ isotype) and etanercept (Enbrel®; Fc-TNFR2 fusion protein).[4] These new

biologic agents have changed the paradigm of RA treatment, leading to improvements in controlling the signs and symptoms of RA refractory to classical low-molecular-weight drugs such as methotrexate (MTX), and in slowing joint destruction.[5] The concentration of golimumab necessary to neutralize TNFα-induced E-selectin expression on human endothelial cells by 50% is reported to be less than those for infliximab and adalimumab.[6] Nevertheless, patients currently treated with an anti-TNFα agent may remain refractory or become nonresponsive to the treatment.[7] In addition, a sizable proportion of patients experience adverse events, such as hepatitis B infection and tuberculosis.[8] Once a primary TNFα inhibitor fails, a patient must switch to another anti-TNFα agent that has a complementary mode of action or differs in its capacity to induce immunogenicity.[9]

The molecular basis underlying the inhibitory mechanisms of infliximab, adalimumab and certolizumab has been elucidated in part by X-ray crystallography.[10, 11, 12] Adalimumab binds a large surface area on TNFα, with its epitope directly overlapping the TNFR2 binding area. Infliximab's epitope is located at the "shoulder" on a bell-shaped TNFα trimer and contains a smaller TNFα:TNFR2 interacting surface. Certolizumab binds to the "waist" portion on TNFα and has overlapping segments with the binding footprint of TNFR2. However, the structural basis for the inhibitory action of golimumab remains unclear. Here, we report the crystal structure of the variable domain (Fv) fragment of golimumab in complex with TNFα at a resolution of 2.73 Å, which reveals an idiosyncratic mode of binding to TNFα to compete with TNFRs at the atomic level.

Results and Discussion

Overall structure of the TNFα:golimumab Fv complex

The soluble forms of the human TNFα and the Fv fragment of the golimumab, both of which contain intrachain disulfide bonds, were produced by a Gram-positive bacterial secretory expression. For production of the Fv fragment, the "iRAT (intervening removable affinity tag)" system was used to ensure stoichiometric expression of heavy (V_H)- and light (V_L)-chain variable domains followed by correct folding and assembly.[13] By employing these recombinant proteins, the TNFα:golimumab Fv complex (subsequently referred to as the TNFα:golimumab complex) was prepared. Analytical gel filtration confirmed that the stoichiometrically saturated 1:3 TNFα homotrimer:golimumab complex (approximately 126 kDa) exists as a monomer in solution (Supplementary Fig. S1A, S1B). The crystals of TNFα:golimumab complex (Supplementary Fig. S1C) appeared in the $P2_1$ space group and diffracted X-rays to 2.73 Å (Table 1). The structure was resolved by molecular replacement using the existing TNFα apo structure (PDB code: 1A8M)[14] and pembrolizumab Fv (PDB code: 5B8C).[15] The asymmetric unit contained two copies of the TNFα homotrimer and six copies of the golimumab Fv, thus providing six copies of the TNFα:golimumab interface related by non-crystallographic symmetry (NCS). The side-chain electron density was sufficiently resolved throughout the TNFα:golimumab interface (Supplementary Fig. S1D and S1E).

The three-dimensional arrangement of the biologically relevant TNFα:golimumab complex has an overall shape that resembles a bell, with the

TNFα trimer and the three Fv fragments sitting at the shoulder of the bell (Fig. 1A, left). In this binding orientation, the golimumab would point away from the membrane in the membrane-bound form of TNFα, with the membrane located below the TNFα molecule in Fig. 1A (left). Therefore, golimumab not only binds the soluble form of TNFα but also may, in principle, be able to bind to TNFα before it is released from its membrane-bound precursor. This structure-based assumption is in line with a pervious pharmacological data that golimumab IgG bind to membrane-bound TNFα with an apparent dissociation constant (K_D) of 1890 pM.[6] When viewed along the intrinsic 3-fold axis of the TNFα trimer, the biologically relevant complex resembles a three-bladed propeller, with each TNFα:Fv protomer forming one blade (Fig. 1A, right). The epitope important for binding and specificity is located within a single TNFα protomer, and the Fv fragments do not bridge protomers in the trimer. The pseudo 2-fold axis of the Fv fragments relating the V_H and V_L domains is not perpendicular to the 3-fold axis, and the Fv fragments are tilted ~45° out of the plane (Fig. 1A). Structural alignment of the TNFα:TNFR2 complex[16] with the TNFα:golimumab complex shows no major perturbations in the TNFR2 binding sites except for the DE loop of TNFα (Fig. 1B). The aberrant positioning of the DE loop in the TNFα:golimumab complex might be caused by a crystallization artifact related to the packing contacts between the peripheral regions of molecules rather than a biologically relevant conformational change caused by binding of golimumab. In the crystals, the DE loop of one TNFα molecule interacts with its neighboring TNFα molecule via hydrogen bonds (Supplementary Fig. S2). Furthermore, it cannot be assumed that the DE loop, which is situated distal to the golimumab

epitope, specifically undergoes allosteric conformational changes even though proximal regions exhibit no apparent shift upon golimumab binding.

Because of the high affinity of golimumab for TNFα, we assume that a stoichiometrically saturated 1:3 TNFα homotrimer:golimumab complex could be formed *in vivo*, although molecular populations of the 1:3 complex might not be dominant, which is similar to the cases of higher-order structures of TNFα:adalimumab and TNFα:infliximab complexes as recently shown by negative stain TEM and cryo-EM techniques.[17] However, formation of the 1:3 complexes is not be necessary for inhibition of signaling via TNFRs because this type of signaling requires multiple intact TNFR-binding sites on the TNFα homotrimer, as demonstrated by inhibition of signaling when TNFα exchanges protomers with dominant-negative TNFα variants.[18]

Golimumab epitope

To establish the residues that fall within the golimumab epitope on TNFα, we analysed the interfaces of the TNFα:golimumab complex via PISA.[19] All NCS copies share the same pattern of intermolecular contacts, which involve 13 TNFα residues and bury surface areas of 905 Å^2 and 997 Å^2 on TNFα and golimumab, respectively (Fig. 2A). These are in the typical range of the interaction surface between antibodies and protein antigens.[20] The golimumab heavy chain contributed 781 Å^2 of the buried solvent-accessible area, whereas the light chain contributed 216 Å^2. The golimumab is bound to a discontinuous epitope composed of residues in the AA' loop (Gly24^T), C strand (Lys65^T, Gln67^T), CD loop

(Ser71^{T}), EF loop (Glu104^{T}, Thr105^{T}, Pro106^{T}, Glu107^{T}, Gly108^{T}, Ala111^{T}) and GH loop (Arg138^{T}, Asp140^{T}, Tyr141^{T}) of TNFα (hereafter, residues of the TNFα and golimumab Fv heavy chain and light chain are designated by the superscript chain identifiers T, H, and L, respectively). Over 18 pairs of interactions are observed, including 11 direct hydrogen bonds between residues, at least 3 water-mediated hydrogen bonds, 3 salt bridges, 1 aromatic side-chain contact and hydrophobic interactions, which indicate a stable interaction network between the two proteins and may explain their high binding affinity (K_D=18 pM when measured using soluble form of TNFα and golimumab IgG).[6] The recognition of the epitope is mainly achieved by the complementarity-determining region (CDR) loops of the heavy chain. Most prominently, residues located in the EF loop of TNFα provide a key component of the interactions, mainly through hydrogen bonds and salt bridges to the CDR-H1 (Ser31^{H}) and CDR-H3 (Arg98^{H}, Arg100^{H} and Tyr111^{H}) of golimumab (Fig. 2B and Supplementary Fig. S2). Arg100^{H} makes the largest contribution to the TNFα:golimumab interaction and presents a binding free energy of -7.7 kcal/mol as calculated with ANCHOR.[21] From the golimumab light chain, only two residues, Tyr30^{L} and Tyr32^{L} in CDR-L1, are involved in the interaction with Gly24^{T} (AA' loop) and Asp140^{T} (GH loop), respectively.

Notably, the TNFα residues interacting with golimumab CDRs are distinct from those that engaged TNFR2 (Fig. 2A and Supplementary Fig. S3). A previous crystallographic study of the TNFα:TNFR2 complex (PDB code: 3ALQ) showed that the cysteine-rich domain 2 (CRD2) and CRD3 of TNFR2 have contacts with residues in the AA' loop (Gln21^{T}, Glu23^{T}, Arg31^{T}, Arg32^{T}, Ala33^{T}) and GH loop

(Asp143^{T}) of one TNFα protomer, and the DE loop (Ser86^{T}, Tyr87^{T}, Pro90^{T}) of a neighbouring protomer[16]. None of these residues participates in polar interactions within the TNFα:golimumab complex; hence, direct competition does not occur between the binding residues of golimumab and TNFR2, which represents an idiosyncratic antagonistic feature exhibited by golimumab that is not shared with the other three anti-TNFα antibodies. The latter three antibody epitopes have overlapping portions with the binding footprint of TNFR2, including buried solvent-accessible surface areas of 26.1 Å^2 for infliximab (Fig. 2C), 211.7 Å^2 for adalimumab (Fig. 2D) and 224.9 Å^2 for certolizumab (Fig. 2E).

TNFR steric competition properties of golimumab

Because golimumab inhibition of TNFR binding could not arise from direct competition for receptor-binding residues on TNFα and/or potential golimumab-induced conformational changes in TNFα, steric clashes caused by physical overlap between the bound golimumab and TNFRs could be substantial and critical to golimumab's inhibitory activity. To gain quantitative insights into the effect of inhibitor overlap on blocking TNFα interactions with TNFRs, we calculated the theoretical volumes of atomic overlap between golimumab and TNFR2. First, we performed a structural alignment of the TNFα:golimumab complex with the TNFα:TNFR2 complex. We then calculated the volumes of atomic overlap between the superimposed golimumab and TNFR2 (Fig. 3). Similar analyses were also performed for infliximab (Supplementary Fig. S4A and S4B), adalimumab (Supplementary Fig. S4C and S4D) and certolizumab

(Supplementary Fig. S4E and S4F). Surprisingly, golimumab exhibits a volume of steric conflict with TNFR2 (30 Å^3) that is an order of magnitude smaller than that of the other three anti-TNFα antibodies (infliximab versus TNFR2=1,800 Å^3; adalimumab versus TNFR2=2,090 Å^3; and certolizumab versus TNFR2=820 Å^3).

Furthermore, the atomic overlapping volumes between anti-TNFα antibodies and TNFR1 were calculated. Because the structure of the TNFα:TNFR1 complex has not been determined, we used the structure of the lymphotoxin-α (TNFβ):TNFR1 complex (PDB code: 1TNR)[22] as a template to generate a model of TNFα:TNFR1 complex, which was then superimposed with the TNFα:antibody complexes. This analysis revealed that significant steric clashes occur between golimumab and TNFR1 (360 Å^3) (Fig. 4), although they are smaller than those of the other three anti-TNFα antibodies (infliximab versus TNFR1=1,820 Å^3; adalimumab versus TNFR1=2,110 Å^3; and certolizumab versus TNFR1=530 Å^3) (Supplementary Fig. S5). This steric conflict between golimumab and TNFR1 underlies, in part, the therapeutic efficacy of golimumab, which has strong inhibitiory effects on TNFα-binding to TNFR1 and presents an IC_{50} of 3.9 ng/mL as previously described.[6]

Interestingly, golimumab's physical overlapping volumes with the two TNFRs are drastically different. Such a disparity is not observed for the other three anti-TNFα antibodies. However, given that even a small overlapping volume between the receptor and the antibody can cause steric repulsion, it is clear that golimumab exhibits competitive inhibitory action against both TNFR1 and TNFR2. Previous functional analyses of TNFRs have demonstrated the predominant role of TNFR1 in the pathogenesis and exacerbation of

inflammation.[23, 24] Moreover, TNFR2 has been shown to be crucial for the proliferation, activation and antigen presentation of T-cells, which are essential in cell-mediated immune responses against bacteria and viruses.[25] Blocking all effect of TNFRs signaling can therefore be counter-productive. Clinical trials have assessed the side effects of golimumab treatment and reported an increased risk of infections, reactivation of tuberculosis, invasive fungal infections, and other opportunistic infections.[26, 27] These adverse drug reactions can be explained in part by our finding that golimumab sterically hinders TNFR2 as well as TNFR1 from associating with TNFα (Figs. 3 and 4).

In summary, structural analysis of the TNFα:golimumab complex and its comparison to the TNFα:TNFR2 complex revealed that the binding footprints of golimumab and TNRF2 on TNFα do not overlap at all; however, steric clashes between the bound golimumab and TNFRs are critical to its inhibitory activity. Golimumab does not induce conformational changes in TNFα that could affect receptor binding. This mode of action of golimumab is different from that of the other currently approved anti-TNFα therapeutic antibodies, infliximab, adalimumab and certolizumab pegol. Our findings may provide structure-based insights to guide engineering efforts to create even more efficient versions of anti-TNFα biologics.

Materials and Methods

Complete amino-acid sequence of the expression constructs

The cloned *Homo sapiens* TNFα sequence contains residues 77 to 233 of the 233 total residues (UniProt accession number: P01375), with the FLAG-tagged at the N-terminus underlined and additional C-terminal residues retained after restriction site cloning or tobacco etch virus (TEV) cleavage shown in italics (refer to the following section for cloning details):

<u>DYKDDDDK</u>VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQL
VVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGA
EAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL*TSENLYFQ*

The golimumab light chain variable region (V_L), with the additional N- and C-terminal residues retained after restriction site cloning or TEV cleavage shown in italics is as follows:

*AGS*EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGI
PARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIK*TSENLYFQ*

The golimumab heavy chain variable region (V_H), with the additional N-terminal residues retained after restriction site cloning or TEV cleavage shown in italics is as follows:

*SKL*QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYD
GSNKKYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYG
MDVWGQGTTVTVSS

Protein expression and purification

The soluble form of TNFα was produced by secretion from the Gram-positive bacterium *Brevibacillus choshinensis.*[13] The artificially synthesized codon-optimized cDNA of TNFα was inserted downstream of and in frame with the secretion signal sequence of the plasmid pNY326 (Clontech). To facilitate the detection and purification of the secreted proteins, sequences for the TEV protease cleavage site and a His_6 tag were placed at the C-termini of the TNFα cDNAs. *B. choshinensis* cells harbouring the TNFα-expression plasmid were grown at 30 °C with shaking at 200 rpm in 2SY medium (soytone 40 g/L, yeast extract 5 g/L, glucose 20 g/L, and $CaCl_2$ 0.15 g/L) supplemented with 50 mg/L neomycin for 65-70 h. The recovered culture supernatant was adjusted to a final ammonium sulfate concentration of 60% saturation. The precipitate was pelleted, dissolved in TBS buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl), and dialyzed overnight against the same buffer. The dialyzed sample was purified with Ni-NTA resin, mixed with TEV-His_6 and dialyzed overnight again against TBS buffer. The cleaved His_6 tag and TEV-His_6 were removed using a HisTrap column. The flow-through fractions were further purified with a HiLoad 16/60 Superdex 75 column (GE Healthcare).

The golimumab Fv was expressed and purified using the iRAT system as previously described.[13]

Crystallization

The protein complex was prepared by incubating TNFα with golimumab Fv at a molar ratio of 1:6 for 1 h on ice. The complex was subjected to size exclusion chromatography (Superdex 200 10/300 column, GE Healthcare). Peak fractions containing the TNFα:golimumab Fv complex were concentrated to approximately 10 mg/mL by ultrafiltration (Millipore, MWCO 50 kDa) and used for crystallization experiments. The crystals of the complex used for structural determination were grown at 20°C by hanging drop vapor diffusion. A 400 μL reservoir containing 0.1 M sodium acetate (pH 4.6) and 1.4 M NaCl was equilibrated against a 2 μL drop containing a 1:1 mixture of the complex and reservoir solution. After 14-21 days of growth, the crystals were cryo-protected in 25% ethylene glycol in the mother liquor and then flash-frozen in liquid nitrogen.

Data collection, structure determination and analysis

The diffraction data were collected at 100 K at the SPring-8 beamline BL41XU (Japan) using a PILATUS3 6M detector. The data were then integrated and scaled using XDS.[28] The structure of the TNFα:golimumab Fv complex was determined by molecular replacement with the program MR-PHASER[29] using the atomic coordinates of the human TNFα apo structure (PDB code: 1A8M) and the pembrolizumab Fv (PDB code: 5B8C) as the search models. The model was further rebuilt in COOT[30] and refined with phenix.refine[31] (Table 1). In the final Ramachandran plot, 95.6% and 4.1% of residues were in the favored and allowed

regions, respectively. The refined structures were visualized with PyMOL (http://www.pymol.org/). The PISA server[19] was used to identify protein-protein interactions and estimate the solvent-accessible surface area. The model complex of TNFα:TNFR1 was constructed based on the structural alignment of the LTα (TNFβ):TNFR1 complex (PDB code: 1TNR)[22] and the TNFα:TNFR2 complex (PDB code: 3ALQ)[16] using a superimposing program in UCSF Chimera.[32] The volumes of physical overlap of each anti-TNFα antibody with TNFRs were calculated by the volumes tool in Chimera.

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Conflicts of Interest Statement

The authors declare no conflict of interest associated with this manuscript.

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Additional Information

Accession numbers: The atomic coordinates and structure factors for TNFα:golimumab Fv complex have been deposited in the Protein Data Bank (http://www.rcsb.org) under accession code 5YOY.

Table 1. Crystallographic data collection and refinement statistics

Protein name	TNFα: golimumab Fv complex
X-ray data collection	
Source, wavelength	SPring-8 BL41XU, 1.00000 Å
Resolution (Å)[a]	46.25-2.73 (2.80-2.73)
Space group	$P2_1$
Unit cell parameter (Å, °)	a=133.6, b=85.3, c=136.0, α=90.0, β=101.6, γ=90.0
Unique reflections[a]	77915 (5439)
Redundancy[a]	3.4 (3.5)
Completeness[a]	97.1 (92.8)
R_{merge} (%)[a]	10.7 (98.8)
$CC_{1/2}$ (%)[a]	99.3 (53.9)
$<I/\sigma(I)>$[a]	9.5 (1.5)
Refinement	
Resolution (Å)[a]	46.25–2.73 (2.76-2.73)
R_{work} / R_{free} (%)[a,b]	19.0 / 23.4 (35.4 / 39.9)
R.m.s. deviations	
Bonds (Å)	0.003
Angles (°)	0.672
No. of atoms (average B-factors (Å²))	
TNFα	7194 (81.8)
golimumab Fv	10836 (75.4)
Water	86 (59.3)
Ramachandran plot	
Favored region (%)	95.6
Allowed region (%)	4.1

[a]Values for the highest resolution shells are shown in parentheses.

[b]R_{work} was calculated with 95% of the unique reflections used for refinement, whereas R_{free} was calculated with the remaining 5% of the unique reflections.

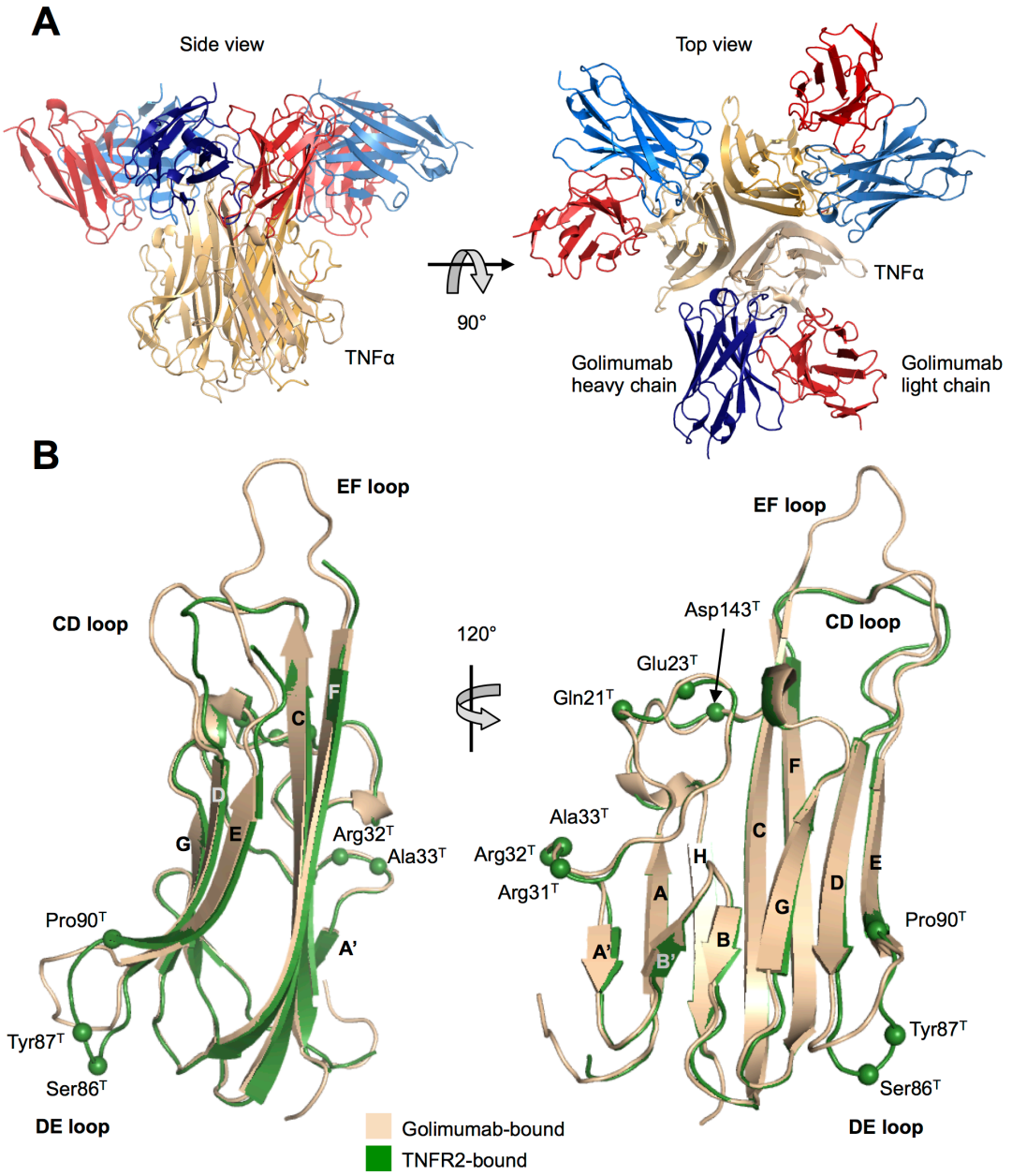


Figure 1. Overall structure of the TNFα:golimumab complex. (A) Ribbon diagrams of the biological unit showing the TNFα homotrimer and three golimumab Fvs binding to each protomer in the side view (left) and top view (right). TNFα, golimumab V_L and golimumab V_H are shown in tan, red and blue, respectively. (B) Structural alignment of the golimumab-bound TNFα protomer (tan) with TNFR2-bound TNFα protomer (green). Canonical designations of β-strands and loops within TNFα are also shown. The TNFα protomer is rotated by 120° around the vertical axis. Residues in TNFα that contact TNFR2 are indicated by green balls.

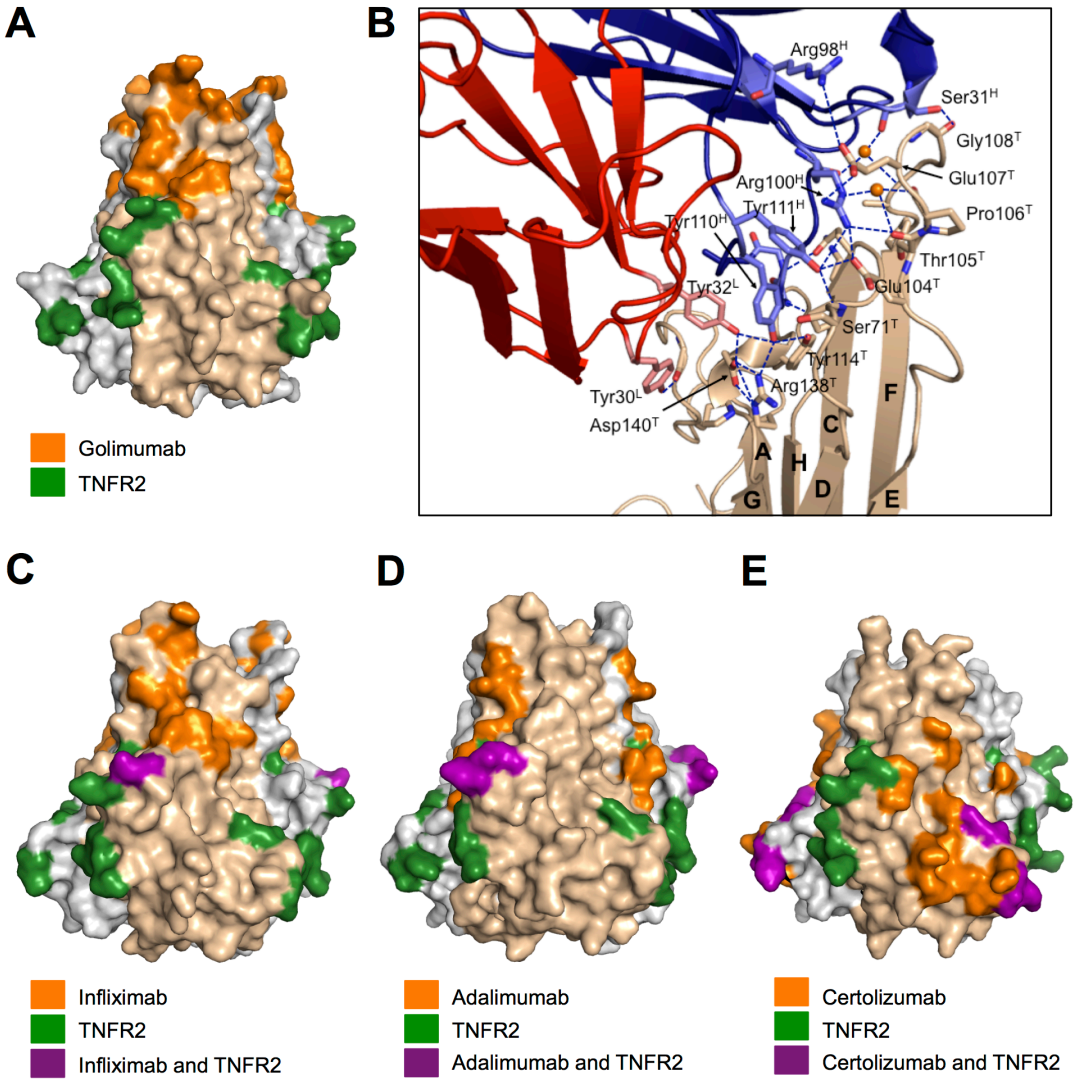


Figure 2. TNFα:golimumab contact residues and comparison with those of the other anti-TNFα mAbs. (A) Surface diagram of TNFα from the TNFα:golimumab complex structure, with the golimumab epitope highlighted in orange. One TNFα protomer is shaded tan, and the other two protomers are shown in grey. The TNFR2-binding sites are outlined in green. (B) Close-up views of interfaces. Residues involved in hydrogen bonds (blue dashes) are shown. The colour-coding is the same as in Figure 1A. Water molecules are shown in orange. Previously published epitopes of infliximab, adalimumab and certolizumab are shown in (C), (D) and (E), respectively. Residues for the polar interactions with anti-TNFα mAbs (orange) and TNFR2 (green) and the overlapping contact residues (purple) are coloured differently.

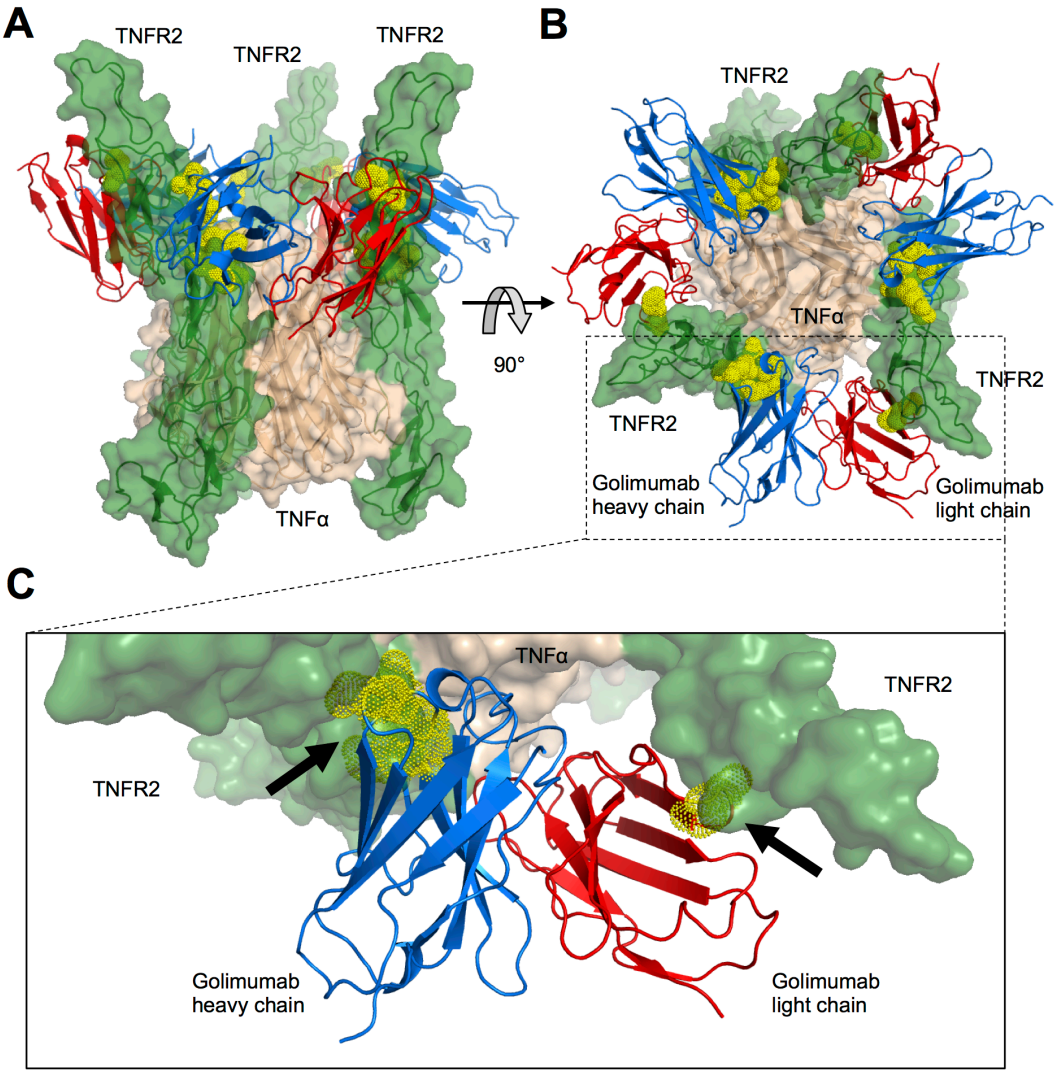


Figure 3. Structural basis of golimumab and TNFR2 competition. Structural alignment of TNFα:golimumab and TNFα:TNFR2 complexes reveals that the golimumab light chain (red) and golimumab heavy chain (blue) penetrate into the volume of TNFR2 (green), which would allow golimumab to block TNFR2 binding. The aligned structure is rotated by 90° around the horizontal axis in (A) and (B). (C) Close-up view of the area shown by the box in dotted lines in (B) showing the steric conflicts (black arrows). Golimumab residues responsible for the steric conflict are shown by dot diagram in yellow.

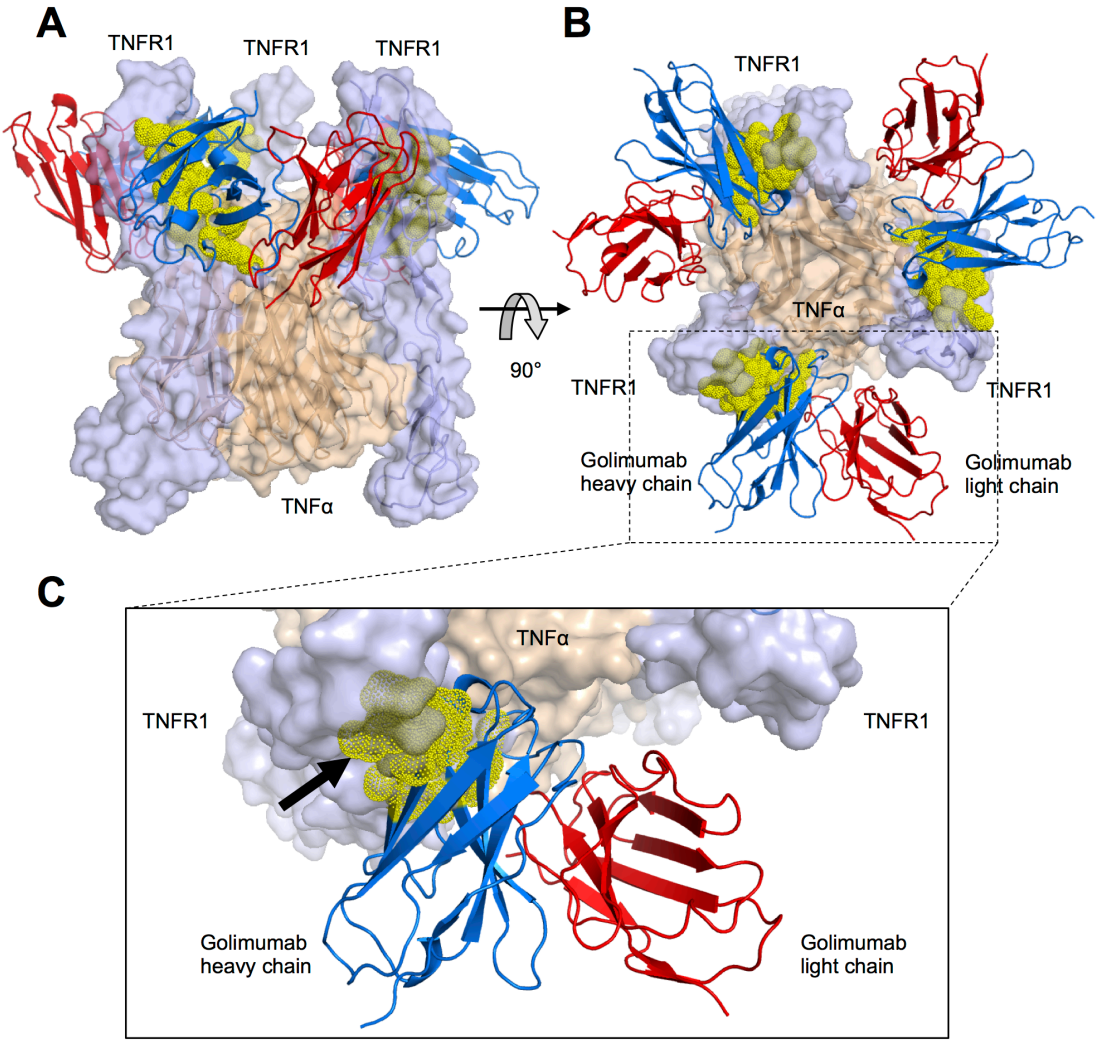
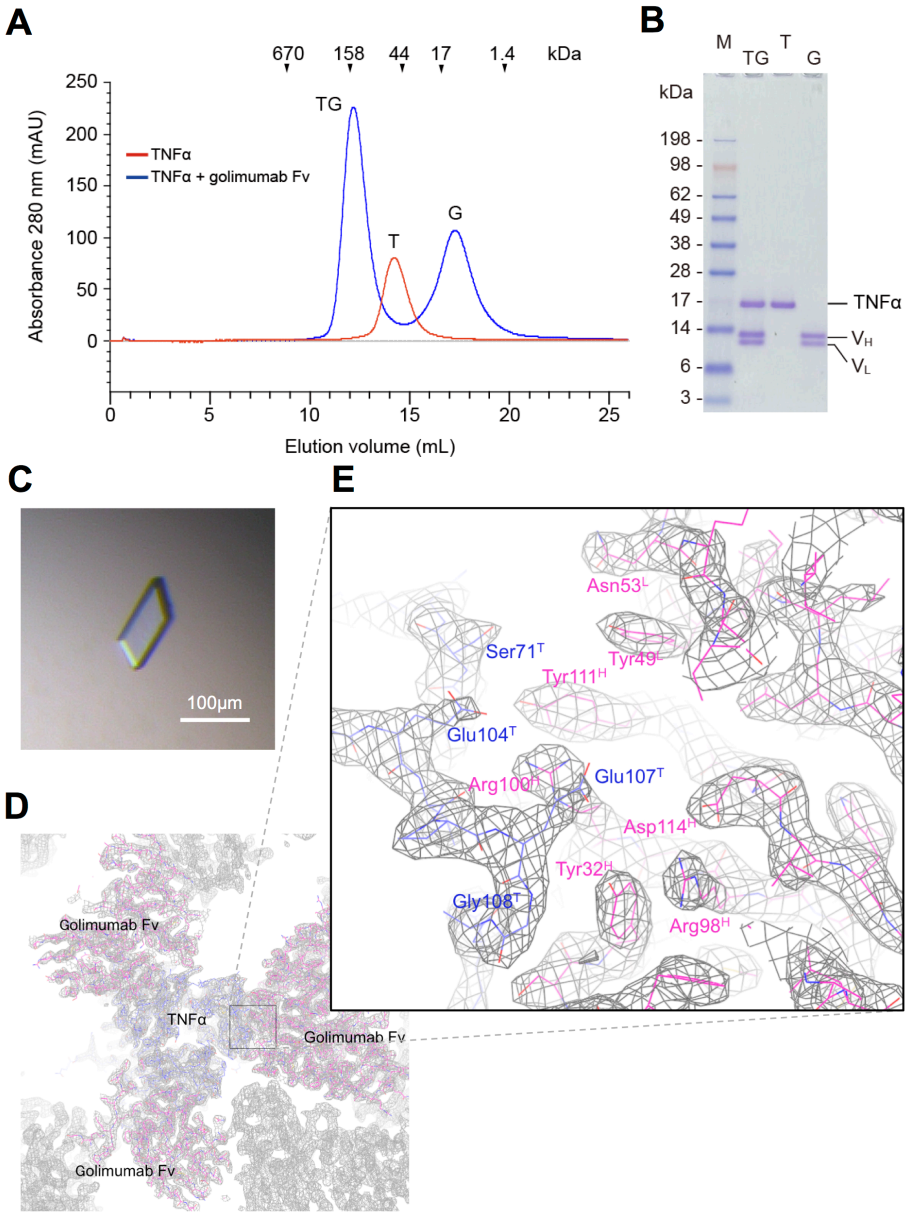
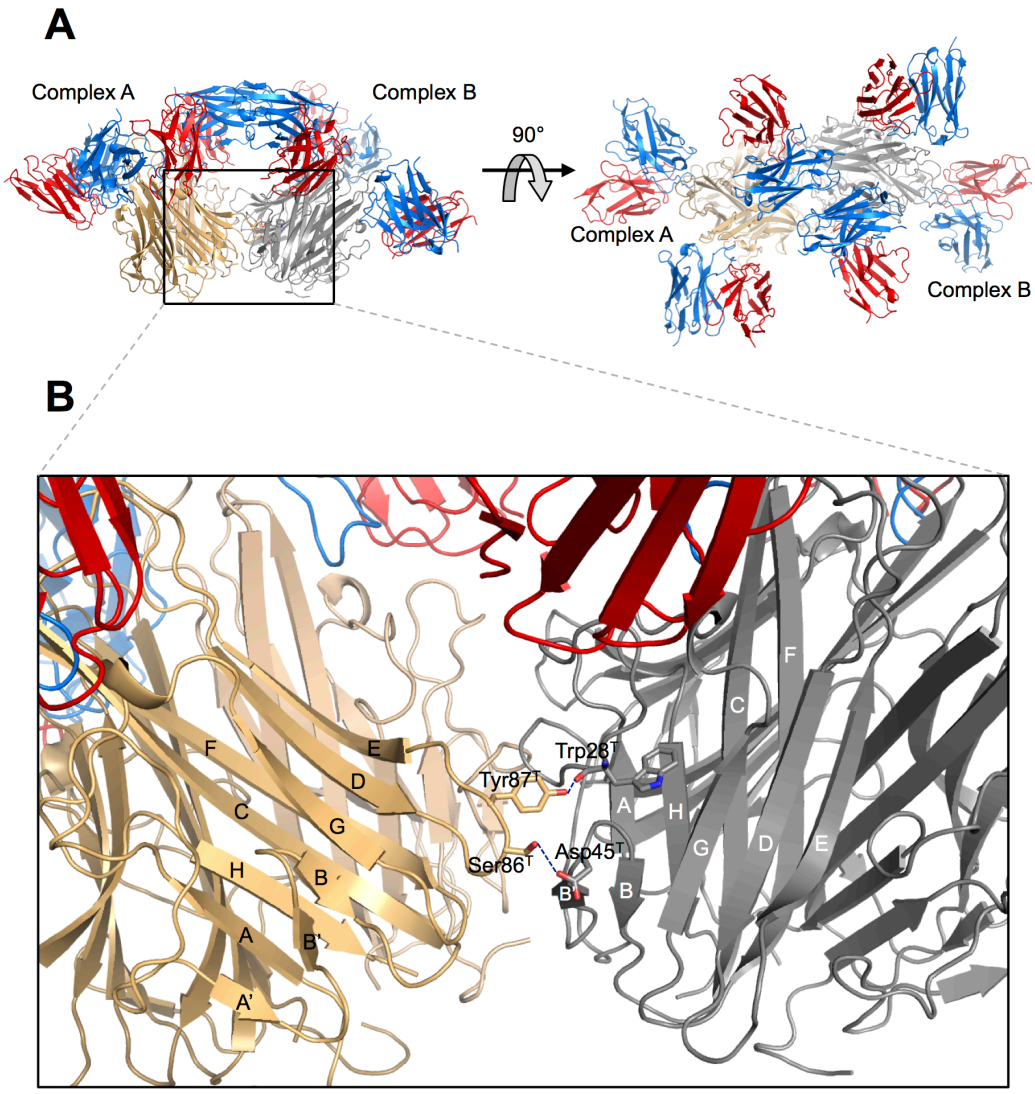


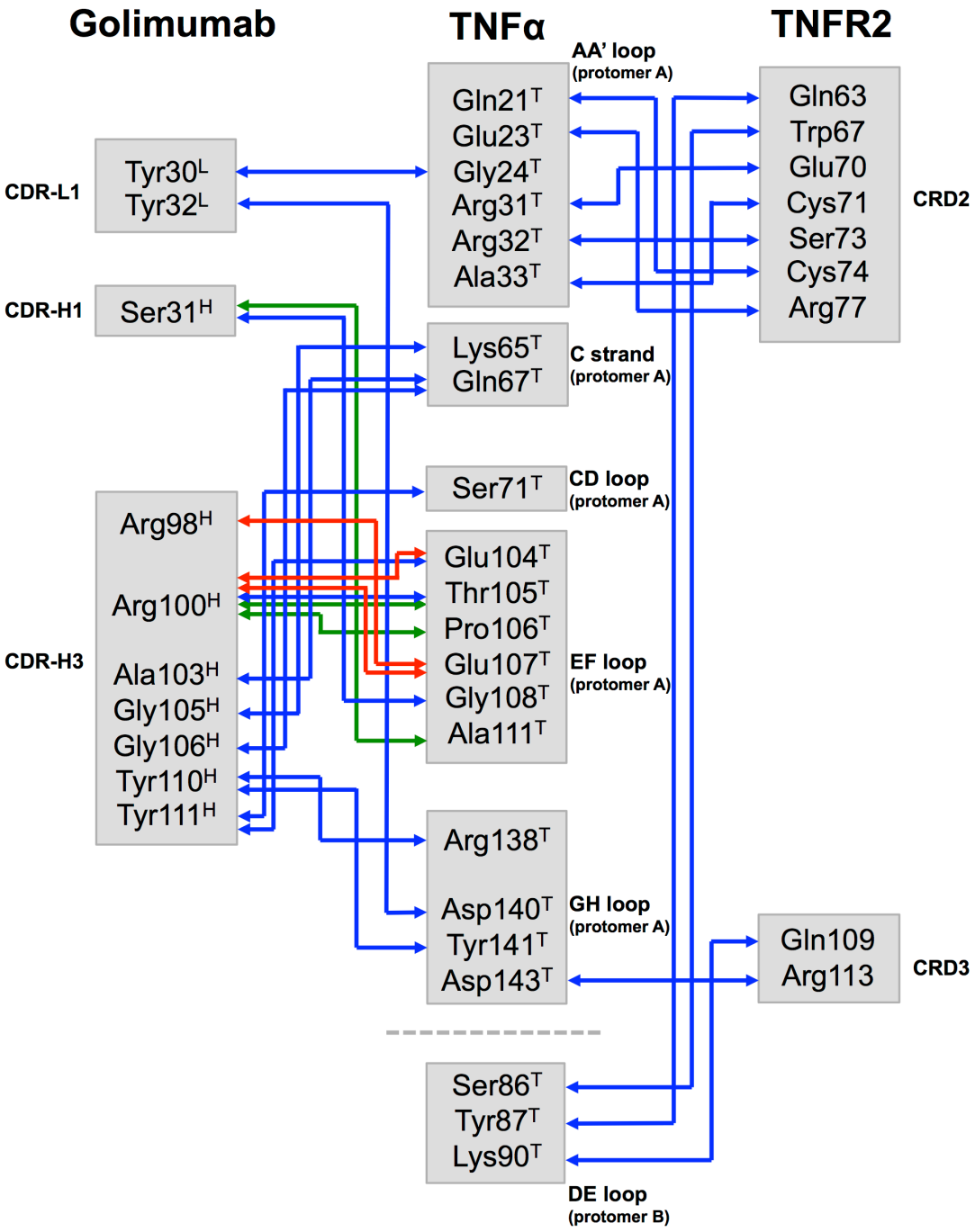
Figure 4. Structural model for golimumab and TNFR1 competition. Structural alignment of the TNFα:golimumab complex and the TNFα:TNFR1 complex model reveals that the golimumab heavy chain (blue) penetrates into the volume of TNFR1 (light blue), which would allow golimumab to block TNFR1 binding. The aligned structure is rotated by 90° around the horizontal axis in (A) and (B). (C) Close-up view of the area shown by the box in dotted lines in (B) showing the steric conflicts (black arrow). Golimumab residues responsible for the steric conflict are shown by dot diagram in yellow.



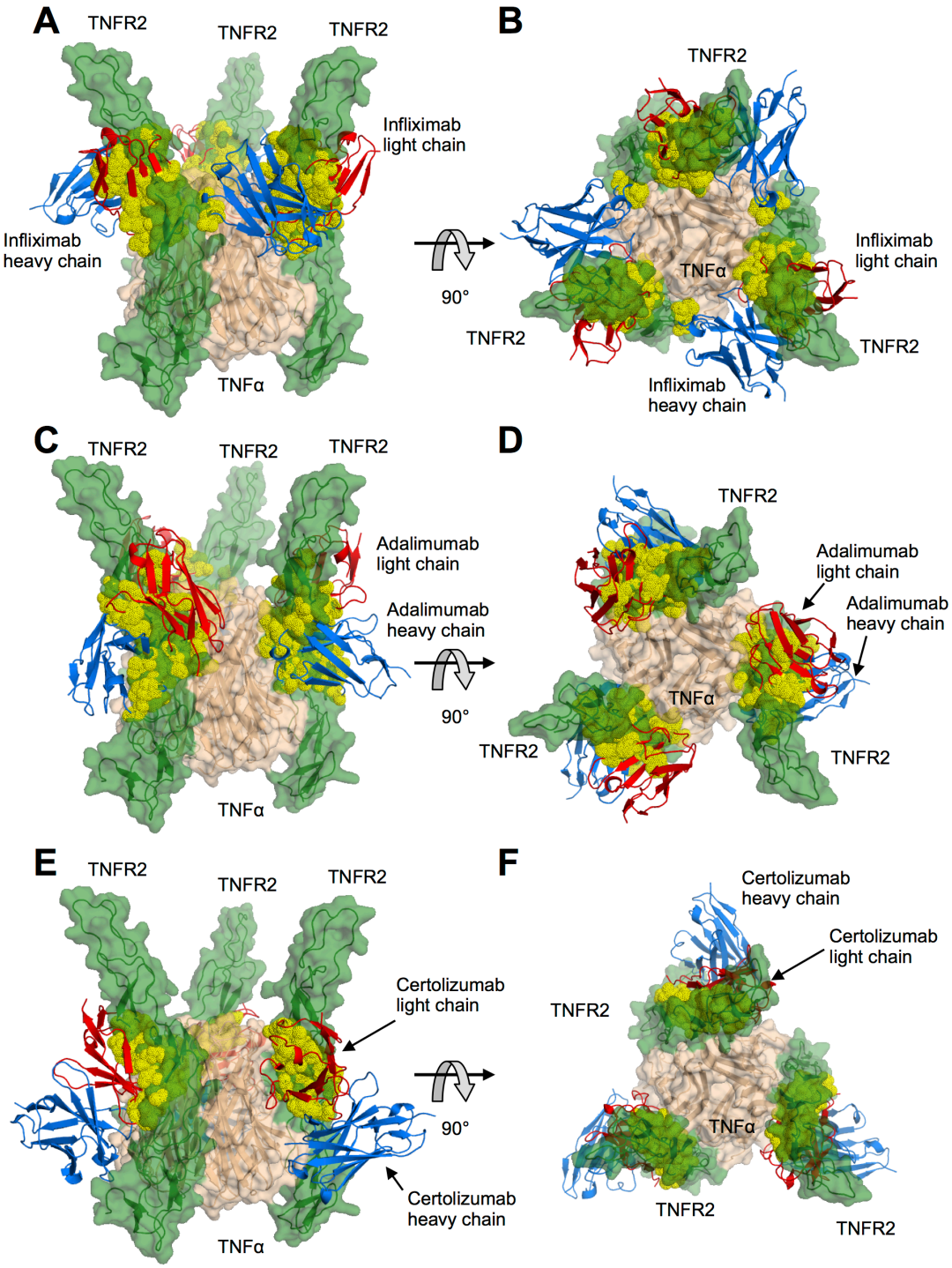
Supplementary Figure S1. Crystallography of the TNFα:golimumab Fv complex. (A) Typical profile in size exclusion chromatography of the TNFα:golimumab Fv complex in the presence of excess golimumab Fv on a Superdex200 10/300GL column (blue line). As a reference, TNFα alone was separated on the same column (red line). Elution volumes of protein standards are indicated at the top. Peak TG: TNFα:golimumab Fv complex; peak T: free TNFα; and peak G: free golimumab Fv. (B) SDS-PAGE analysis of the corresponding peak fractions in (A). (C) Crystal of the TNFα:golimumab Fv complex. (D) Representative portions of the 2*mFo-DFc* electron density map (1.2σ) for an overall model of the TNFα:golimumab Fv. (E) Close-up of the interface; residues of TNFα and golimumab are numbered in light pink and blue, respectively.



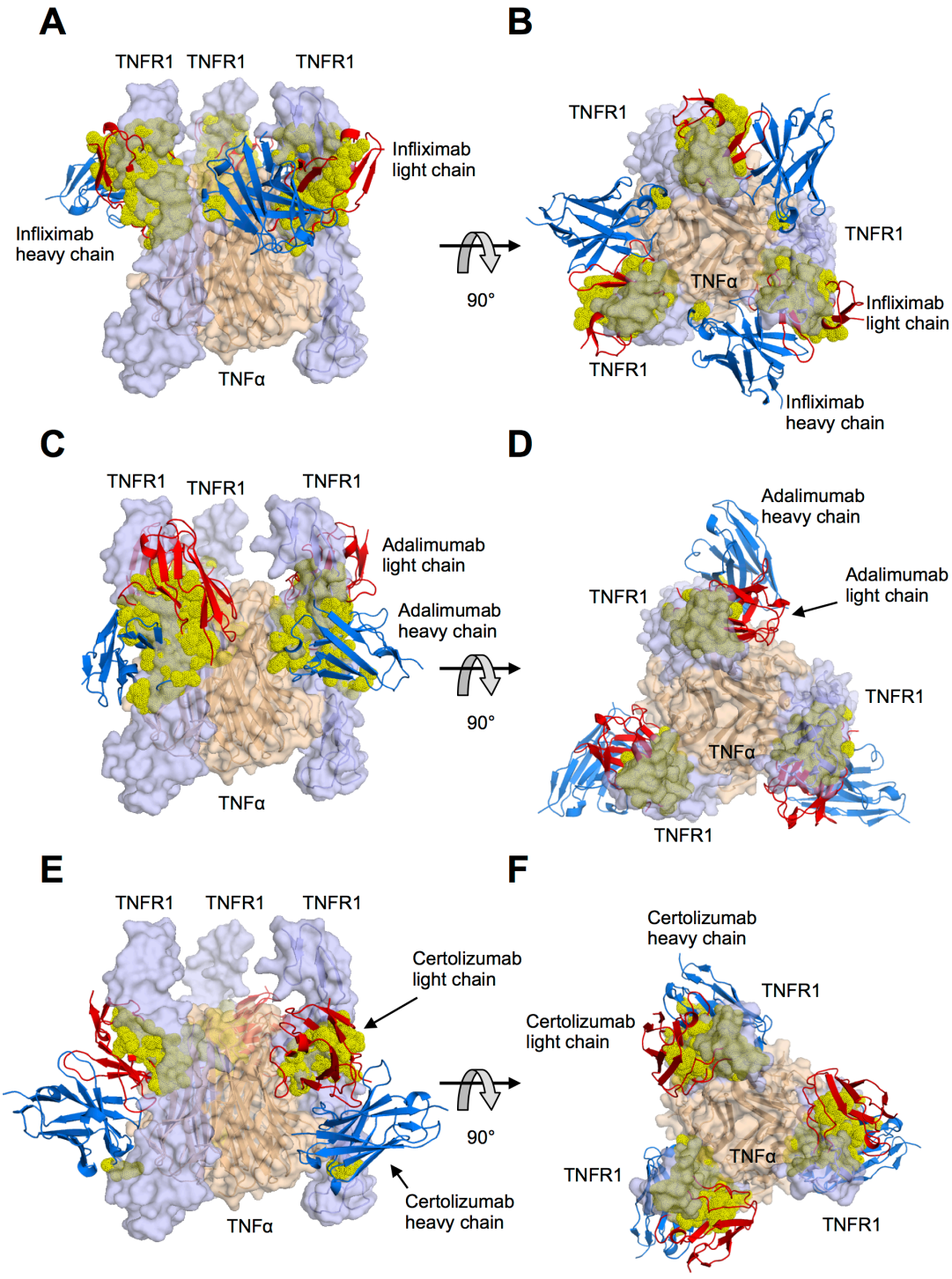
Supplementary Figure S2. The DE loop is involved in crystal packing interactions. (A) Riboon representation of two TNFα:golimumab Fv complexes in the asymmetric unit. Golimumab V_L and golimumab V_H are shown in red and blue, respectively. TNFα in complexes A and B are shown in tan and grey, respectively. (B) Close-up view of interactions between the two complexes. $Ser86^T$ and $Tyr87^T$ in the DE loop of one TNFα molecule (complex A) interact with $Asp45^T$ and $Trp28^T$ of the other TNFα molecule (complex B), respectively. Dotted lines connect atoms within hydrogen-bond distances.



Supplementary Figure S3. A schematic diagram of polar interactions. Direct protein/protein hydrogen bonds are blue; water-mediated hydrogen bonds are green; and salt bridges are red.



Supplementary Figure S4. Competitive binding between FDA-approved anti-TNFα mAbs and TNFR2. Structural alignment of TNFα:mAb and TNFα:TNFR2 complexes reveals that the mAb light chain (red) and mAb heavy chain (blue) have a significant penetration into the volume of TNFR2 (green), which would allow the mAbs to block TNFR2 binding. The aligned structures are rotated by 90° around the horizontal axis. Infliximab is shown in (A) and (B); adalimumab is shown in (C) and (D); and certolizumab is shown in (E) and (F).



Supplementary Figure S5. Models for competitive binding between FDA-approved anti-TNFα mAbs and TNFR1. Structural alignment of TNFα:mAb complex and TNFα:TNFR1 complex model reveals that the mAb light chain (red) and mAb heavy chain (blue) have a significant penetration into the volume of TNFR1 (light blue), which would allow the mAbs to block TNFR1 binding. The aligned structures are rotated by 90° around the horizontal axis. Infliximab is shown in (A) and (B); adalimumab is shown in (C) and (D); and certolizumab is shown in (E) and (F).